A SEARCH FOR THE VECTOR OF *PLASMODIUM PTEROPI* BREINL.

By A. J. Bearup and J. J. Lawrence, School of Public Health and Tropical Medicine, University of Sydney.

[Read 31st July, 1946.]

Introduction.

Following on the discovery of malaria parasites in man similar parasites were found in the red blood cells of bats and other animals. It was quickly shown that anopheline mosquitoes were the invertebrate hosts of human plasmodia, but no vector has yet been found for the plasmodia of bats, nor have the details of their development in the vertebrate host yet been elucidated.

Plasmodium pteropi Breinl (1913) was first described from the flying fox, *Pteropus gouldi* Peters in north Queensland, where it is common in these animals. Human malaria, mostly due to *Plasmodium vivax*, also occurs in parts of this area, *Anopheles punctulatus farauti* Laveran (= *moluccensis* Sw. & Sw. de Graaf) being the vector (Heydon, unpublished data). Surveys of the infection rate in "wild-caught" mosquitoes would be liable to error if anopheline mosquitoes were vectors of *P. pteropi* as well. The immediate object of the present work was to attempt to infect anopheline mosquitoes with *P. pteropi*, and if this was unsuccessful, to extend the work in the hope of finding the vector of this parasite.

Historical.

From Italy, Dionisi,* in 1899, described *Polychromophilus melanipherum* from *Miniopterus schreibersii* and *P. murinus* from *Vespertilio murinus*. He failed to find any segmenting stages of these parasites but as they were pigmented and intracorpuscular they are at present classified in the genus *Plasmodium*. He also described an unpigmented parasite, *Achromaticus vesperuginus*, from *Vesperugo noctula*; this is probably a piroplasm. Three species of mosquitoes, *Anopheles claviger*, *Aëdes caspius* (= *Culex penicillaris*) and *Aëdes vexans* (= *Culex malariae*) were fed by him on infected bats but none became infected.

The next important contribution is that of Schingareff (1907), who described segmenting stages of *P. murinus* in the peripheral blood, liver and spleen of *Vespertilio daubentoni* but only gametocytes of *P. melanipherum* from *M. schreibersii*. Since his bats always harboured wingless flies of the family Nycteribiidae he dissected six, but found no evidence of infection. These observations were made in Russia.

Vassal (1907), working in Annam, found *Vesperugo abranus* infected with a parasite which he described as *Plasmodium melanipherum* var. *monosoma*. He fed the mosquitoes *Culex pipiens* and *Anopheles subpictus* (= *Myzomyia rossii*) on the bat and dissected them from one to ten days later with negative results.

In 1913, Breinl described *Plasmodium pteropi* from *Pteropus gouldi* in north Queensland, the first record of a plasmodium from a flying fox (Megachiroptera) in Australia. A similar parasite was described by Mackie (1914) in *Pteropus edwardsii* in India; in ignorance of Breinl's prior use of the name, he also called it *Plasmodium pteropi*. In any case the two appear to be identical; the figures show rings, gametocytes and forms which are described as "segmenters". Mackie kept an infected flying fox in a cage with uninfected animals none of which developed the infection, although all were infested with Nycteribiidae. Dissections of some of these flies were without positive result.

Rodhain (1926) described *Plasmodium epomophori* from the epauletted flying foxes of the Belgian Congo; in natural infections gametocytes were always present but

* Quoted from Manwell, 1946.

schizonts were seldom found and no mature segmenters were ever seen. In captivity the infected foxes were at times attacked by *Cimex lectularius*, none of which developed an infection; neither did *Aëdes aegypti* (= *Stegomyia fasciata*) nor species of *Culex* which he dissected several days after a blood feed. There were no permanent ecto-parasites such as Nycteribiidae on these foxes. In contrast to this, Rodhain found the common flying fox, *Eidelon helvum*, to be always infested with Nycteribiidae but never to be infected with plasmodia. In fact, it proved refractory to infection by blood inoculation.

Materials and Methods.

At night, flying foxes range the countryside in search of food, which consists of the blossoms of various trees and fruit, both wild and cultivated, but during the day they congregate in "camps" and rest hanging from the topmost branches of trees. Two such camps were located; one, a mixture of *Pteropus gouldi* Peters and *P. scapulatus* Peters, was in a mangrove swamp on Magnetic Island, near Townsville; and the other, of *Pteropus conspicillatus* Gould only, in a tea-tree swamp on the outskirts of Cairns. In both camps there were young unweaned flying foxes, *P. gouldi* and *P. conspicillatus* respectively (*P. scapulatus* differs in its breeding season from the two former species). If Ratcliffe's (1931) estimation of the month of birth of these species be correct, the ages of the young *P. gouldi* would be one to two months, and of the *P. conspicillatus* two to three months. Those we classed as adults were at least twelve months older.

A very high proportion of the adults of all three species were infected with *Plasmodium pteropi*, nearly all showing gametocytes in the blood. Of the young *P. gouldi*, 9 of 17 were infected, and of the young *P. conspicillatus*, 3 of 23. (See Table 1.) The actual number of positives would be higher than these figures show, as in most cases they are based on a single examination. In the captive animals light infestations sometimes fail to show parasites even in thick films. *Pteropus scapulatus* and *P. conspicillatus* are new host records for *Plasmodium pteropi*.

Table 1.

Frequency of Infections in Flying Foxes.

Species.	Adult.		Young.	
	Positive.	Negative.	Positive.	Negative.
Pteropus gouldi	25	0	9	8
P. scapulatus	10	1	0	0
P. conspicillatus	15	3	3	20

These infections in young animals made it probable that active transmission of the parasite was taking place, so in order to get an idea of what blood-sucking insects were flying about, collections were made in the camps, using man as a bait; some of these insects were later dissected and examined for evidence of infection. If the infection of foxes was taking place in the camp it was thought that a day-biting insect must be responsible. However, we found that the camps were not entirely deserted by night as many of the young were left hanging in the trees and a few adults were always about; this made it necessary to collect at night, too.

Some young infected *Pteropus gouldi* were caught and kept in captivity to be used in attempts to infect mosquitoes. These foxes were shown to be potentially infective by the demonstration of gametocytes in stained films and occasionally by the observation of exflagellation of male gametocytes in blood diluted with saline.

The mosquitoes were usually reared from larvae or pupae collected in the field but in some cases "wild-caught" adults were used. The *Anopheles punctulatus punctulatus* Dönitz were from stock originally sent from New Guinea and which had been reared through many generations in the laboratory. They were from a colony regularly used for the experimental transmission of *Plasmodium vivax* and *P. falciparum* infections.

The flying fox was immobilized by tying it to a board and it was then placed in the mosquito cage for an hour. This method gave fair results with all the species of mosquitoes except *Culex fatigans* Wiedemann. Slightly better results were obtained with this species by allowing the mosquitoes to feed overnight on the infected flying fox

which was confined in a small cage beneath a mosquito net. The fed mosquitoes were collected and kept for periods up to 20 days, some being dissected at intervals. The air temperature was roughly 80°F. and the humidity high. In most cases both salivary glands and midgut were examined.

An attempt was made to feed some "wild-caught" sand-flies of the genus *Culicoides* on an infected animal but this was a failure.

Results.

The numbers of the different species of mosquitoes dissected after having fed on infected flying foxes are given in Table 2. Usually both midgut and salivary gland were examined. None showed any evidence of infection.

Table 2.

Dissections of Mosquitoes fed on Infected Bats.

Species of Mosquito.	Number Dissected.
Anopheles punctulatus punctulatus	125
Anopheles punctulatus farauti	3
Anopheles annulipes	4
Aëdes vigilax	54
Aëdes aegypti	27
Aëdes notoscriptus	34
Aëdes funereus	28
Culex annulirostris	28
Culex sitiens	5
Culex fatigans	26

It has been mentioned previously that collections of winged biting insects were made, using man as a bait. Day-biting insects found in the mangrove swamp were *Aëdes vigilax* Skuse, *Culex sitiens* Wiedemann, *Culicoides* sp., and *Tabanus* sp. The *Culicoides* sp. has been stated by Lee to be near *C. molestus* Skuse, but perhaps a distinct species. The only night-biter collected was *Culex sitiens*. In the tea-tree swamp, *Aëdes vigilax*, *Culex annulirostris* Skuse, and *Aëdes funereus* Theobald were present by day; and at night these and *Aëdes kochi* Dönitz also. Some of these "wild-caught" insects were examined for evidence of infection (midgut and usually salivary glands as well) but none were positive. The species and their numbers were as follows: *Culicoides* sp., 12; *Aëdes kochi*, 8; *Aëdes vigilax*, 6; *Culex sitiens*, 18; *Tabanus* sp., 3.

Flying foxes of the three species which we examined (*Pteropus gouldi*, *P. conspicillatus* and *P. scapulatus*) were all parasitized by Nycteribiidae, identified by Lee as *Cyclopodia albertsii* Rond. (= *Cyclopodia pteropus* Rainbow). A number were collected from living and dead flying foxes, which had gametocytes in their blood. In all, forty-nine *Cyclopodia*, about equal numbers of each sex, were dissected, and the midgut, and in almost all cases, the salivary glands as well, were examined for evidence of infection. All examinations were negative.

Discussion.

If a mosquito be the vector of this plasmodium it is surprising that no positive result came from the series of experimental feedings shown in Table 2. With the human plasmodia, under laboratory conditions, practically all species of anophelines are susceptible to infection, although in nature most of them take no part in the spread of the disease. It seems most unlikely that anophelines can act as the invertebrate host, especially *A. punctulatus punctulatus*, which is an important vector of human malaria in New Guinea. It seems probable that active transmission of the plasmodium was taking place in the camp in the mangroves at Magnetic Island where many of the sucklings were already infected. Although flying foxes shift camp fairly frequently, this particular one had been occupied for at least a month before our arrival, and as the food supply in the surrounding districts was plentiful, it is not likely that the flying foxes, encumbered with young, had been engaging in migrations. It is probable that the young flying foxes were not only born but also infected in the district. Of the day-biting insects found here it seems possible to exclude *Aëdes vigilax* and perhaps also

Culex sitiens as vectors, for although we did not dissect many of the latter, it is probable that one of the other *Culex* species would have proved susceptible had this been the vector.

Some other species of mosquitoes such as *Aëdes funereus* and *Culex annulirostris*, common in close association with the camps of these animals, also seem to be excluded as vectors.

Culicoides is common enough in mangrove swamps but we made no satisfactory test of its susceptibility. It would be worth while considering as a possibility in any future work.

We have consistently failed to find satisfactory evidence of the presence of schizonts in the blood or organs of the bats we have examined. Both Breinl and Mackie figure and describe schizonts but these could have been male gametocytes. Recent observations on this point have come from Manwell (1946) who examined blood and organ smears of flying foxes (*Pteropus gouldi* and *Dobsonia moluccensis*) from New Guinea. From several of the blood smears of *P. gouldi* he describes extracellular segmenters, devoid of pigment, which in some cases resemble the exo-erythrocytic forms of bird malaria. He considers that what has been regarded as a species of *Plasmodium* may be, in reality, more closely allied to *Haemoproteus*, and, as a corollary of this, that it would be more logical to look for vectors among the Nycteribiidae and Streblidae.

Our results with the nycteribiid, *Cyclopodia albertsii*, do not support this suggestion. In addition to the negative results of our dissections there is other indirect evidence against this possibility. We have several times found infected mothers with uninfected sucklings though these mothers had *Cyclopodia* on them. Mackie had a similar experience. Also, as has been mentioned, he kept an infected *Pteropus edwardsii* in a cage with other flying foxes but none of the latter became infected although nycteribiids were present.

Summary.

1. In a search for vectors of *Plasmodium pteropi* the mosquitoes listed in Table 2 were fed on infected *Pteropus gouldi*. No positive evidence of infection of midguts or of salivary glands was obtained. These observations seem to show that mosquitoes, especially anophelines, are unlikely to be important vectors of this parasite.

2. Dissections of *Cyclopodia* removed from infected animals were also negative.

3. *Pteropus scapulatus* and *P. conspicillatus* are recorded as new hosts for *Plasmodium pteropi*.

Acknowledgements.

Permission to undertake this investigation was granted by the Director-General, Commonwealth Department of Health, Canberra, and by the Director, School of Public Health and Tropical Medicine, Sydney. Of the many persons who gave assistance special mention is made of Dr. G. A. M. Heydon, Parasitologist to the School; the Senior Commonwealth Medical Officer, Brisbane; the Medical Officers in Charge of the Health Laboratories at Townsville and Cairns; Lieut.-Col. C. R. Bickerton Blackburn and Major M. J. Mackerras of the Malaria Research Unit at Cairns; Mr. R. Norris of the C.S.I.R. Buffalo Fly Investigation Unit at Malanda and members of their staffs.

Mr. D. J. Lee, Zoology Department, University of Sydney, gave invaluable help in the determination of entomological material.

References.

Breinl, A., 1913.—Report for the year 1911, Australian Institute of Tropical Medicine, Townsville.

Mackie, F. P., 1914.—Note on the Parasite of Bat Malaria. *Ind. J. Med. Res.*, 2 : 375.

Manwell, R. D., 1946.—Bat Malaria. *Amer. J. Hyg.*, 43 : 1.

Ratcliffe, F. N., 1931.—The Flying Fox (*Pteropus*) in Australia. *Counc. Sci. Industr. Res.*, Melbourne, Bull. No. 53.

Rodhain, J., 1926.—*Plasmodium epomophori* n. sp. parasite commun des Roussettes épaulières au Congo belge. *Bull. Soc. Path. exotique*, 19 : 828.

Schingareff, A. J., 1907.—Des Hémosporidies des chauves-souris. *Arch. Sci. biol.*, 12 : 181. (Quoted from Manwell above.)

Vassal, J. J., 1907.—Sur un hématozaire d'un chéiroptère. *Ann. Inst. Pasteur*, 21 : 224.